IJFSNPHT

ISSN: 0975 – 8712
IJFSNPHT (2011), 3(1):1-3

Microbiology of Fresh Oranges after Storage at Room and Refrigeration Temperature Conditions

Ahmad Allafi[1], Saad Busamri[2]

1 Assistant Professor, Department of Family Sciences, College for Women, Kuwait University, P.O.Box: 5969, Safat 13060, Kuwait. Tel: (965)2498-3161, Fax: (965)2251-3929

ahmadallafi@yahoo.com

2 Lecturer, Nutrition and Food Science Department, The Public Authority for Applied Education & Training, Kuwait

ABSTRACT:
The objectives of this study are to determine whether storage temperatures affect the microbial count and to determine what types of microbes grow on oranges. Oranges were homogenized by stomaching or being placed on an orbital shaker. Dilutions for each sample were plated both on acidified potato dextrose agar and tryptic soy agar. It was determined that storage temperature had little effect, if any, on the overall microbial counts of the samples.

Keywords: Food Safety, Microbiology, Bacteria, Kuwait, Orange, Preservation, Refrigeration

INTRODUCTION

There are multiple uses of oranges. They are a rich source of vitamins and minerals. They can be used in the preparation of juice, squash concentrated juice, and juice powder or eaten as is. Their citrus oil and peel oil can also be extracted. They also have good medicinal value [1].

There are several possible sources of contamination that may damage fruit that may occur at many different points during growth, during the handling processes and after distribution. The soil, insects, and animals could all contaminate during growth period of oranges. The water used for irrigation or washing during the processing could be contaminated and then sprayed onto the oranges. Temperature abuse and inadequate washing during processing, shipping to grocers, and handling of oranges by the employees and customers at the store may also contribute to contamination. Contamination may spoil fruit to the point that it is unappealing to eat. Many fruits have rinds or peels that can keep many microorganisms from infesting and proliferating, such as oranges. It has been documented however, that those microbes can be transferred from the rind to the inner flesh, which could be harmful to the individual eating the damaged fruit [2].

As it turns out, the flesh of an orange is not completely sterile of microbes. There is a natural opening in the rind at the point of the navel, the stem-end vascular tissue, where infiltration of solutions is possible. There is a great chance that microorganisms are included in these solutions that are able to get inside the orange. The key time when this infiltration occurs is during the hydrocooling of the produce after picking. Research has shown that *E. coli* O157:H7 is able to infiltrate fresh produce no matter what its temperature happens to be. It is also noted that dipping produce in tanks of hot water so as to kill any insects only aids in the survival of these infiltrating pathogens. Also, the dyes used to color fresh produce have the ability to penetrate through the rind. Some microorganisms are able to get into the peel along with the dye; however, the dye is able to get through holes that are too tiny for most microbial cells which restrict greatly the number and kinds of microbes that can infiltrate along with the dye [3].

Orange and other citrus trees are subject to a great number of fungal diseases affecting the roots, the trunk and branches, the foliage and the fruits. Greasy spot, caused by *Cercospora citri-grisea*, is seen, 2 to 9 months after severe infection, as yellow-brown, blistery, oily, brown or black spots on the foliage. Severe defoliation may follow. The fungus, *Diaporthe citri, is* responsible for gummosis, melanose, dieback and stem-end rot [4]. The fungus, *Elsinoe australis*, causes sweet orange scab which is frequently seen on oranges in South America and in Sicily and New Caledonia. *Phytophthora megasperma*, *P. palmivora* and *P. Parasitica* are also common causes of food rot. Another typical fungus is known as the *Rodotorula* spp., which is characterized by reddish, budding yeast [5]. *Rhizopus* spp., is also a very common food spoilage fungus and is characterized by a large sporangiospore with striated walls and a nonseptate root-like structure. *Fusarium* spp. is also found growing on oranges and other citrus fruits and is characterized by sickle-shaped multicelled, macroconidia with foot cells. Each of these microorganisms will proliferate in and on oranges in especially damp conditions. The standard levels of microorganisms that are considered acceptable for fruits are $10^3 – 10^5$ CFU/g [6].

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Blue mold rot or storage rot is the most feared storage disease of citrus fruits and is caused by two species of mold: green mold (*Penicillium digitatum*), which is of an olive-green color, and blue mold (*Penicillium italicum*), which is of a blue-green color. The fungal spores mainly penetrate through small injuries and initially form white, circular spots of fungal growth, which are subsequently covered from the center outwards with a green or blue-green sporulating layer. The peel becomes spongy, the pulp soft – a typical instance of wet rot. Development is optimal at 20 - 27°C; growth still flourishes at 10°C and comes to a standstill only below 4°C. Blue mold is transferred from fruit to fruit by contact. Seawater, rain and condensation water promote green and blue mold growth. The name *Penicillium* comes from the word penicillus which means a brush and this is based on the brush-like appearance of the fruiting structures under the microscope. Millions of dollars worth of losses are caused each year by these fungi during storage or transit of oranges. *Penicillium* is a versatile, opportunistic fungus with an arsenal of useful enzymes at its disposal to attack a host of organic foodstuffs [5].

Due to all different kinds of contamination to which produce is susceptible, one could transfer enough microbes to can cause illness. Therefore, the objective of this study is to assess the microbial activities of oranges after storage at room and refrigeration temperatures.

MATERIALS AND METHODS

A total of eight oranges were split into two groups of four based on their storage temperature - refrigerated and room temperature. All oranges that remained intact, that is, unpeeled were then shaken on an orbital shaker in 99 ml of peptone water for 2 minutes. All oranges that were peeled were then stomached in 99 ml of peptone water for 2 minutes. A dilution series was then performed on each; 10^{-2}, 10^{-3}, 10^{-4} for those that were peeled and 10^{-1}, 10^{-2}, and 10^{-3} for those that remained unpeeled in 9 mL peptone blanks. 0.1 mL of each of the last three dilutions was then double spread plated on both the Acidified PDA and the TSA as to gain a better average for the total growth. A total of 48 plates were then incubated at 30ºC for 5 days to maximize the growth of yeasts and molds which grow at a fairly slow rate. After this incubation period, each plate was enumerated to determine the CFU/orange. Then the colony and cell morphology were assessed and presumptive microorganisms were compiled.

Cell morphology was observed using a light microscope and different staining techniques including: lactophenol cotton blue dye, Gram stain, and simple staining using crystal violet. Also, a phase contrast microscope was used to observe some fungi and bacteria.

RESULTS AND DISCUSSIONS

According to Table 1, the samples that were peeled displayed higher microbial counts. These higher counts indicate that it is probable that some of the microbial load from the rind was transferred to the flesh during the peeling process. The reason it was suspected that a transfer of microbes from rind to flesh occurred was due to the observation of similar colonies on the plated samples of both the peeled and unpeeled oranges. For example, almost all plates contained fuzzy green colonies presumably tested to be *Penicillium* spp.

At refrigeration temperatures fungi and some bacteria, including psychrotrophs, survive and proliferate. At room temperatures, on the other hand, other bacteria and some fungi can survive and proliferate. According to Table 1, it was determined that storage temperature had little effect, if any, on the overall microbial counts of the samples. This was due to counting total microbes grown as opposed to counting the fungi and bacteria separately. However, more mold growth was visibly observed on the refrigerated samples' plates.

Table 1. Counts of yeast and mold (and possibly acid tolerant bacteria) in oranges as determined through the use of shaker/stomacher homogenization then plated on Acidified PDA and TSA and incubated at 30ºC

Orange Conditions	Dilutions	Acidified PDA[a]		TSA[b]		Average Count (CFU/orange)[c]
		Plate 1	Plate 2	Plate 1	Plate 2	
Unwashed Unpeeled Warm	10^{-1}	90	188	196	270	**PDA** $1.4x10^4$ **TSA** $2.0x10^4$
	10^{-2}	8	14	23	45	
	10^{-3}	<1	2	3	3	
Unwashed Unpeeled Cold	10^{-1}	97	267	175	193	**PDA** $9.7x10^3$ **TSA** $2.3x10^4$
	10^{-2}	29	28	29	28	
	10^{-3}	2	5	7	1	
Unwashed Peeled Warm	10^{-2}	>300	>300	>300	>300	**PDA** $9.7x10^5$ **TSA** $7.0x10^5$
	10^{-3}	98	96	68	42	
	10^{-4}	12	19	6	4	
Unwashed Peeled Cold	10^{-2}	5	6	7	2	**PDA** $5.5x10^3$ (est.)[d] **TSA** $4.5x10^3$ (est.)
	10^{-3}	<1	<1	1	1	
	10^{-4}	<1	<1	<1	<1	

[a] Potato dextrose agar.
[b] Tryptic soy agar.
[c] Results are presented as colony forming unit (CFU)/orange.
[d] No sampled plate counts in acceptable countable range.

A plethora of microorganisms were able to grow on and in oranges and a few examples are presented in Table 2. Many of the predicted microbes based on previous research findings were presumed to be found on the samples plated. *Penicillium* spp. was, by far, the most common, presumptively found microbe. Its characteristics include a blue-green rot of citrus fruits and it is able to produce antibiotics. Common yeasts

that were presumptively found were *Rhodotorula*, *Saccharomyces*, *Cryptococcus*. *Rhodotorula* spp. characteristics are: multilateral budding, it is a nonfermenter of sugars and colonies often contain orange or pink color pigmentation and are sometimes mucoidal. *Saccharomyces* spp. also display multilateral budding, have spherical spores, does not ferment lactose, and has white or cream colonies with a yeasty odor. *Cryptococcus* spp. characteristics are: multilateral budding and it is a nonfermenter of sugars [6]. Bacterial species were also presumptively found, including *Acinetobacter*. Other species that can grow on oranges include *Lactobacillus plantarum* and *Enterobacter agglomerans*. *L. plantarum* is classified as a lactic acid bacterium [5]. Therefore, It was concluded from this study that storage temperature did not have a significant effect on the microbial count of oranges. Furthermore, *Penicillium* spp. was the most common found microbe on all samples.

Table 2. The colony and cellular morphologies of samples taken from the oranges tested and resulting presumptive microorganism

Media Sampled From	Colony Morphology	Cellular Morphology	Presumptive Microorganism
Acidified PDA[a]	1 – Fuzzy, large raised, green with white edge, sizes ranging 1 – 3 cm	Approximately 10um in size[b], long, septated hyphae, with many conidiophores, blue[c]	*Penicillium* spp.
	2 – Pink, smooth, shiny, entire, raised, approximately 3 mm	Budding yeast, cocci, purple[d], approximately 4 um in size	*Rhodotorula* spp.
TSA[e]	1 – Fuzzy, white with black center, raised, approximately 1 cm	Long, non-septated hyphae, with a dark ball on the end, approximately 5 um in size	*Rhizopus* spp.
	2 – Fuzzy, light pink, raised with vein-like surface, approximately 2 cm	Sickle-shaped, multicelled macroconidia, approximately 3 um in size	*Fusarium* spp.
	3 – White, entire, gooey with a dense center, approximately 2 cm	Gram-negative, pairs of coccobacilli, approximately 1 um in size	*Acinetobacter* spp.

[a] Potato dextrose agar.
[b] Size when viewed under microscope at 100x with oil immersion.
[c] Blue color from stain with lactophenol cotton blue dye.
[d] P Purple color from simple stain with crystal violet.
[e] Triptic soy agar.

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